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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL SCREENING OF 10 MEMBERED TETRAAZAMACROCYCLIC COBALT COMPLEX AGAINST DRUG RESISTANT BACTERIAL PATHOGENS

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ABSTRACT

Unsymmetrical macrocyclic CoII complex was synthesized by the template process using bis(benzil)ethylenediamine as precursor. Bis(benzil)ethylenediamine (L) reacts with cobalt(II) chloride and hydrazine HCl in a 1:1:1 molar ratio in methanol to give solid metal complex of the general composition [M(L)X2] (where, M = CoII, L = macrocyclic ligand and X = Cl). The precursor and the metal complex of the macrocycle have been physico-chemically characterized by melting point, elemental analyses, molecular weight determinations, molar conductances, IR and electronic spectral studies. The synthesized complex was evaluated for in-vitro antibacterial activity against the multidrug resistant pathogens such as Staphylococcus aureus, Escherichia coli, Shigella Flexneri, Pseudomonas aeruginosa and Vibrio cholerae. The metal complex showed the significant antibacterial activity compared with commercial antibiotics. Further work can be extended through In-silico docking of these complexes and bacterial genome which can be given a better idea about genome metal interaction in molecular level.

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INTRODUCTION

The co-ordination chemistry of macrocyclic ligands is a fascinating area which has attracted the attention of many inorganic chemists. Tetraazamacrocycles and their metal complexes have been studied extensively in connection with their resemblance to naturally

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occurring porphyrins. Saturated macrocycles with various numbers of ring members have been synthesized regularly. These compounds have produced interesting information concerning both the stabilities and structures of their metal complexes (Fujiwara et al., 1990).

The novel approach to macrocyclic synthesis, therefore, overcomes the usual limitations of the more traditional template routes where the metal tends to be "locked" into the macrocycle with demetallation often resulting in decomposition (Bashall et al., 1990). The influence of variation of the structureal feature on metal complexation, namely the length of the alkyl bridge linking the benzil nitrogen atoms, was investigated (Atkinson et al., 1996). Increasing the length of this bridge is expected to increase the 'lopsidedness" of the N_4-donor set. In this way, it was intended not only to increase the available macrocyclic cavity size, but also to introduce increasing "lopsidedness" into the larger members of this series. The use of metal ions as templates in such reactions has led to the synthesis of many metal complexes of macrocyclic ligands (Chandra et al., 1995). These ligands display a number of features of chemical interest. They are relatively rigid in nature and thus impose a specific coordination geometry on the metal ion (Eggleston et al., 1980).

In this paper, we report the synthesis and characterization of new bivalent transition metal complexes from the reaction of bis(benzil)ethylenediamine with hydrazine hydrochloride which show the marked effect of any build-up of ligand strain on the coordination geometry of the central metal. Due to emerging multidrug resistance to antibiotics like ampicillin, chloramphenicol etc., treatment of infection becomes a problem during outbreaks of disease (Brusch, 2009; Dutta et al., 2008; Saha et al., 2006). Currently, the broad spectrum antibiotics were the preferred drugs of choice in many Asian countries, due to increased indiscriminate use of antibiotics for the treatment of humans and animals diseass, thereby developing multidrug resistance organisms, which pose serious threats in developing countries like India (Mijovic & Terzic, 2012). With this view, the synthesized complexes were tested against human pathogenic bacterial isolates for evaluating their antibacterial properties.

METHODS

Melting points were determined in capillary tubes and are uncorrected. All the reagents used in the preparation of macrocyclic ligand and their metal complex was of reagent grade (Merck). The solvents used for the synthesis of macrocyclic ligands and metal complex were distilled before use. All other chemicals were of AR grade and used without further purification.

The microelemental analysis was carried out using CHN-Rapid Analyser. Conductivity was measured on a Toshniwals conductivity bridge using dip type platinised platinum electrode. Molecular weights were determined by the Rast camphor method. The magnetic moments were measured out by using gouy balance. IR spectra (KBr) of the sample was recorded on a shimadzce FTIR-8400s spectro-photometer. The electronic spectra (chloroform) were recorded on the Lamda 35 spectrometer

2.1 Synthesis of the Complex

To a methanolic solution of the precursor complex (Radhakrishna Reddy et al., 1996), bis (benzil) ethylenediamine, hydrazine hydrochloride (4.3mmol) and cobalt chloride

(4.3 mmol) in methanol (25ml) were added and the resulting mixture was heated under reflux for 5-6 h. The solution was cooled and transferred to an evaporating dish and set aside overnight at room temperature. The solid product that separated out was collected, washed with hot water, then with cold methanol and dried.

2.2 Biological Activity

2.2.1 Bacterial cultures Used

The microorganisms used as test organisms are bacteria isolated from clinical samples. One gram positive *Staphylococcus aureus* and four gram negative *Vibrio Cholerae, Shigella Flexneri, Pseudomonas aeruginosa* and *Escherichia coli.* The test organisms were secluded from the clinical samples from patients attending Government Hospital, Kanchipuram, Tamilnadu, India.

2.2.2 Antimicrobial Testing by Disc Diffusion Method

Antimicrobial properties of the synthesized metal complex have been estimated as per the method depicted earlier (N. Prasannabalaji et al., 2012). The organisms were isolated in nutrient agar medium and selectively cultured at 37°C for 24hrs. The bacterial strains were identified by biochemical and standard antibiogram tests as per the directions from Berge's manual for determinative bacteriology. Circular disc of 6mm diameter were prepared from the Whatman No. 1 filter paper. Discs were soaked with equal volume (50µl) of each metal complexes dissolved in DMSO at a concentration of 100µg/ml. The discs were aseptically placed over plates of Muller Hinton agar (MHA, Difco) seeded with each of test pathogens, and the inoculums were adjusted to 0.5 McFarland turbidometry. The plates were incubated in a vertical position at 37°C for 24 hours and the zone of inhibition was calculated (in mm diameter). Inhibition zones with diameter less than 12mm were considered to have low antibacterial activity. Diameters between 12 and 16mm were considered to be moderately active, and those with diameters > 16mm were considered to be highly active. The clinical strains were also tested for their sensitivity against the standard antibiotics, Ampicillin (10mcg), nalidixic acid (10mcg), Methicillin (10mcg) by the disc diffusion method. The solvent control (DMSO) was also maintained throughout the experiment.

2.2.3 Determination of Minimum Inhibitory Concentration (MIC)

The prepared complex is subjected to microbiological testing by using standard strains of *Staphylococcus aureus*, *Vibrio cholerae*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli*. Their activities are compared with the parent compounds. The efficacy is determined by the MIC values.

The complex sample was separately dissolved in the DMSO and Vortexed vigorously under aseptic condition.The MIC assay was performed aseptically in a sterile U bottom 96 well microtitre plate in the laminar hood. In order to determine the minimum inhibitory concentration (MIC), a serial dilution of complexes (2.56mg/ml, 1.25mg/ml, 0.625mg/ml, 0.312mg/ml, 0.15mg/ml, 0.078mg/ml, 0.039mg/ml, 0.019mg/ml and 0.009mg/ml) was prepared and 100µL nutrient broth bacterial suspension was also added to get a final concentration of 0.5 McFarland OD (10^8 CFU/ml). The above procedure of serial dilutions is repeated to obtain preparation for the sample. At the same time, solvent control, positive and negative controls are also run along with the test. The positive and negative controls are free from drug and solvent control were free from macrocyclic complex. Only the positive control wells were inoculated with 1 full loop of the broth culture. After inoculation, the dilutions are incubated at 37°C for 24 hours. After incubation of 24hrs, the turbidity (growth) was checked in the microtitre plates for MIC value. The highest serial dilution which showed no turbidity was taken as the MIC value of the compound.

RESULTS

The physical and the analytical data of the complex were listed in Table **1**. The complex was coloured. The percentage yield and the melting points of the complexes are also given in this Table. The elemental analysis showed the stoichiometry of the metal complex. The complex was highly soluble in chloroform, acetonitrile, DMF and DMSO. In other common organic solvents they are sparingly soluble.

3.1 Molar Conductance Measurements

The molar conductivity values of 10^{-3}M solutions of the complexes in acetonitrile are exhibited in Table 1. The value indicated non-electrolyte nature of this complex. It was also recognized that the chloride ions are coordinated to metal ions, indicating that they are ligands and not simple ions.

3.2 Infrared Spectral Studies

The IR spectra of the complex were interpreted by comparing with the ligand in order to characterize their structures and mode of coordination of ligand.

At 1772cm^{-1} a strong band is observed in the IR spectra of ligand, L, which is assigned to C=O stretching mode of vibration. The appearance a new strong absorption band in the complex lie in the range 1636cm^{-1} which is attributable to the characteristic stretching frequencies of the imino linkage (Shakir et al., 1995) ν(C=N) and the absence of stretching and deformation frequencies of the amino and the carbonyl groups in the IR spectra of these macrocyclic complexes, provide strong evidence for the presence of cyclic product.

The strong as well as sharp band in the range of 1167 was due to the ν(C-N) vibration. The medium intensity absorption bands in the range of 3306cm^{-1} was attributed to ν(C-H) vibration. The band characteristic of the benzil moiety appeared in the range of 1440cm^{-1} (ν_{asym} C_6H_5) and 1317cm^{-1} (ν_{sym} C_6H_5). An important feature is the appearance of a new medium intensity band in the range of 423cm^{-1}, attributed to ν(M-N) which provide strong evidence for the involvement of nitrogen in coordination (Coltrain et al., 1981).

In the far IR spectra of the complex, additional bands are observed in the region of 340cm^{-1} region assigned to γ M-Cl vibrations which validates the participation of chloride in coordination.

3.3 Electronic Spectra

The electronic spectra of bis(benzil)ethylenediamine and its derivatives have been recorded on a Lamda 35 spectrometer. The absorption maximum at 375nm in the case of bis(benzil)ethylenediamine may be assigned to the n→π* transition of the azomethine group. The shift of this band (15nm) in the spectra of the complex indicated the coordination of the nitrogen to the metal atom (Malik et al., 1983). In addition to this band, the spectra of the metal complexes exhibit bands around 270nm and 335nm due to π→π* electronic transitions. However, the position of these bands remains almost unchanged on complexation.

3.4 Antimicrobial Activity

The ligand and the complex were evaluated against different species of bacteria like *Staphylococcus aureus, Vibrio cholerae, Shigella flexneri, Pseudomonas aeruginosa* and *Escherichia coli* at 30 ± 2°C. DMSO was served as a control. Generally, the macrocyclic complex is more active than the open chain derivatives. The antimicrobial assay values by disc diffusion and minimum inhibitory concentrations of the ligand and the complexes were expressed in Tables 2 & 3.

The Complex exhibited best antimicrobial activity against *Escherichia coli* (26mm), *Vibrio cholera* (27mm) and *Shigella flexneri* (20mm). It also showed some bactericidal activity against *Pseudomonas aeruginosa* (13mm) and *Staphylococcus aureus* (11mm).

The mechanism of the toxicity of the complex with the ligands may be ascribed to the increase of the lipophilic nature of the complexes arising from chelation. Due to the complexity of biological system, it is rather difficult to stipulate the exact mechanism for such activities. However, the increased biocidal properties after complexation can be very well explained by chelation theory (Hay et al., 1994, Chaudhary et al., 2001). Antimicrobial drugs are designed to inhibit/kill the infecting organisms and to have nominimal effect on the recepient.

This study authenticates the bactericidal activity of the synthesized Co(II) complex. This complex possesses potent and broad-spectrum antimicrobial activity which could be utilized in the production of pharmaceutically important therapeutic antibiotics.

DISCUSSION

The precursor and the metal complex of the macrocycles have been physico-chemically characterized by melting point, elemental analyses, molecular weight determinations, molar conductances, IR and electronic spectral studies. The synthesized complex was evaluated for in-vitro antibacterial activity against the multidrug resistant pathogens such as *Staphylococcus aureus, Escherichia coli, Shigella Flexneri, Pseudomonas aeruginosa and Vibrio cholerae*. Among the pathogens, *Escherichia coli, Shigella Flexneri and Vibrio cholerae* were highly susceptible to the metal complex. The metal complex showed the significant antibacterial activity compared with commercial antibiotics. This complex possesses potent and broad-spectrum antimicrobial activity which could be utilized in the production of pharmaceutically important therapeutic antibiotics. Thus the cobalt complex can be used as good drug of choice to manage the bacterial diseases after evaluating the *in vitro* effect of metal complex on experimental animal and clinical trials.

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APPENDIX

Table – 1: *Physico-Chemical Characterization of Cobalt Metal Complex*

Complex	**Colour**	**Mol. Wt.**	**M.Pt. (°C)**	**Yield (%)**	**Elemental Analysis % Found (Calculated)**				**$\mu_{eff.}$ BM**	**Molar Conductance (Ω^{-1}cm^{2}mol^{-1})**
					C	H	N	M		
$C_{30}H_{26}CoN_4Cl_4$	green	612	174	62	53.87 (53.59)	4.85 (4.72)	9.17 (9.14)	8.74 (8.60)	4.91	38.92

Table – 2: *Zone of Inhibition (in mm) of Ligand, Cobalt Complex and Commercial Drugs*

Ligand / Complex/ commercial drugs	**Zone of inhibition in mm**				
	Vibrio cholerae	***Shigella flexneri***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Escherichia coli***
Ligand	--	--	--	--	--
$C_{30}H_{26}CoN_4Cl_4$	27	20	13	11	26
Ampicillin (10µg)	31	29	32	29	30
Nalidixic acid (10µg)	28	28	18	23	32
Methicillin (10µg)	-	-	-	9	-

Table – 3: *Minimum Inhibitory Concentration Assay of the Ligand and the Complex*

Ligand / Complex	**MIC at µg/ml**				
	Vibrio cholerae	***Shigella flexneri***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Escherichia coli***
Ligand	--	--	--	--	--
$C_{30}H_{26}CoN_4Cl_4$	16	32	NA	NA	4